BIOLOGICAL EVALUATION
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A NEW INSECT-FUNGAL COMPLEX OF JACK PINE AT BESSEY RANGER DISTRICT, NEBRASKA NATIONAL FOREST

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ABSTRACT

Investigations were undertaken to determine the causal agent(s) of widespread mortality in regenerating jack pine clearcuts on the Bessey Ranger District of the Nebraska National Forest near Halsey, Nebraska. Approximately 40-60% of the advanced regeneration in most cut areas was dead or dying. Symptomatic trees had resinous roots, extensive insect activity on the roots and often on the stem, and a bluestain-like pattern in the roots and lower bole. The stain was identified as the pathogen *Leptographium terebrantis*, or a closely related species in the *Leptographium terebrantis* group (see Zambino & Harrington, 1992). *Dendroctonus valens* (red turpentine beetle) and *Hylastes* sp. were possible vectors collected from pitfall traps under symptomatic trees. The fungus was successfully isolated from *Hylastes* sp. Pathogenicity tests in 2-0 jack pine resulted in 8% mortality, and *L. terebrantis* was reisolated from two symptomatic seedlings. The investigation concluded *L. terebrantis*, a potential pathogen on jack pine, is being spread primarily by *D. valens* and *Hylastes* sp. and predisposing trees to insect feeding by many different species. Suggestions for future management of jack pine stands emphasize reducing breeding material for vectors and planting more resistant species.

INTRODUCTION

The uniqueness of the new insect-fungal complex described here is second only to the uniqueness of the area. Bessey Ranger District, located in the Nebraska sandhills, is part of the Nebraska National Forest system that was planted at the turn of the century with a variety of conifers (Bates & Pierce, 1913; Hunt, 1965). Jack pine (*Pinus banksiana* Lamb.) was the major species planted which was successful, followed by ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), various juniper species, and Austrian pine (*Pinus nigra* Arnold). Planting began in 1903 and continued for over 30 years. The forested area today covers an area greater than 100 square miles.

Jack pine stands today at Bessey are mature and have some of the best growth of the species in the country. However, many stands are approaching 90 years of age, a time when natural jack pine stands tend to break up.

Regeneration of this forest has become a current concern for the land managers. Partly for this reason, a series of small cuts, approximately 30 acres or less, were initiated in the 1980's. Because advanced regeneration was generally quite sparse, most areas were roller-chopped to enhance regeneration and/or planted with jack pine seedlings. The plantings failed for unknown reasons and roller-chopping did not increase regeneration. A few years after the cuts were initiated, the advanced regeneration began to die. Forest Health Management personnel were consulted, and a summary of the results are presented here.

When the plantation areas that had been cut were visited in the spring of 1992, mortality of the advanced regeneration was in the 40-60% range, and mortality was extending to the adjacent mature jack pine stands, as well as causing some mortality in mature Austrian pines. A series of evaluations were initiated at that time to determine probable causal agents and to develop appropriate management recommendations.

A mysterious 'decline' of jack pine on the Bessey Ranger District was reported in the late 1970's and early 1980's (James, 1979; Sharon, 1984). Mature trees were dying in pockets; the symptomology was similar to root disease but no disease was found. *Ips* beetles, staining, and resinosus of the lower bole were often found on affected trees. The staining had been attributed to a species of *Ceratocystis*. The complex found in the dying regeneration which is described in this report closely parallels the situation found in mature stands over a decade ago that had remained a mystery for the most part. Results of this study should shed some light on the declines seen in the past on this jack pine forest.

METHODS

Dozens of jack pine and Austrian pine that were dead and dying, as well as not yet displaying symptoms of infection, were examined in each of the harvested areas shown in Figure 1. Between 5 to 10 root systems of bordering symptomatic mature trees were excavated partially as well, and one tree was dissected with a chainsaw.

In order to isolate the blue stain-like fungus that was found inside the roots of affected trees, a medium selective for *Leptographium* sp. was used: a weak malt agar (1.0%) with 200 ppm cycloheximide and 100 ppm streptomycin. Isolations were made directly from roots collected in the field and from root-feeding beetles collected from pitfall traps in affected areas. Beetles were either soaked in a 10% hypochlorite solution for 20 minutes before plating or were allowed to wander directly on the selective medium. Beetles were also reared on jack pine twigs without any previous evidence of staining in order to observe if beetles were transferring any stain onto wood.

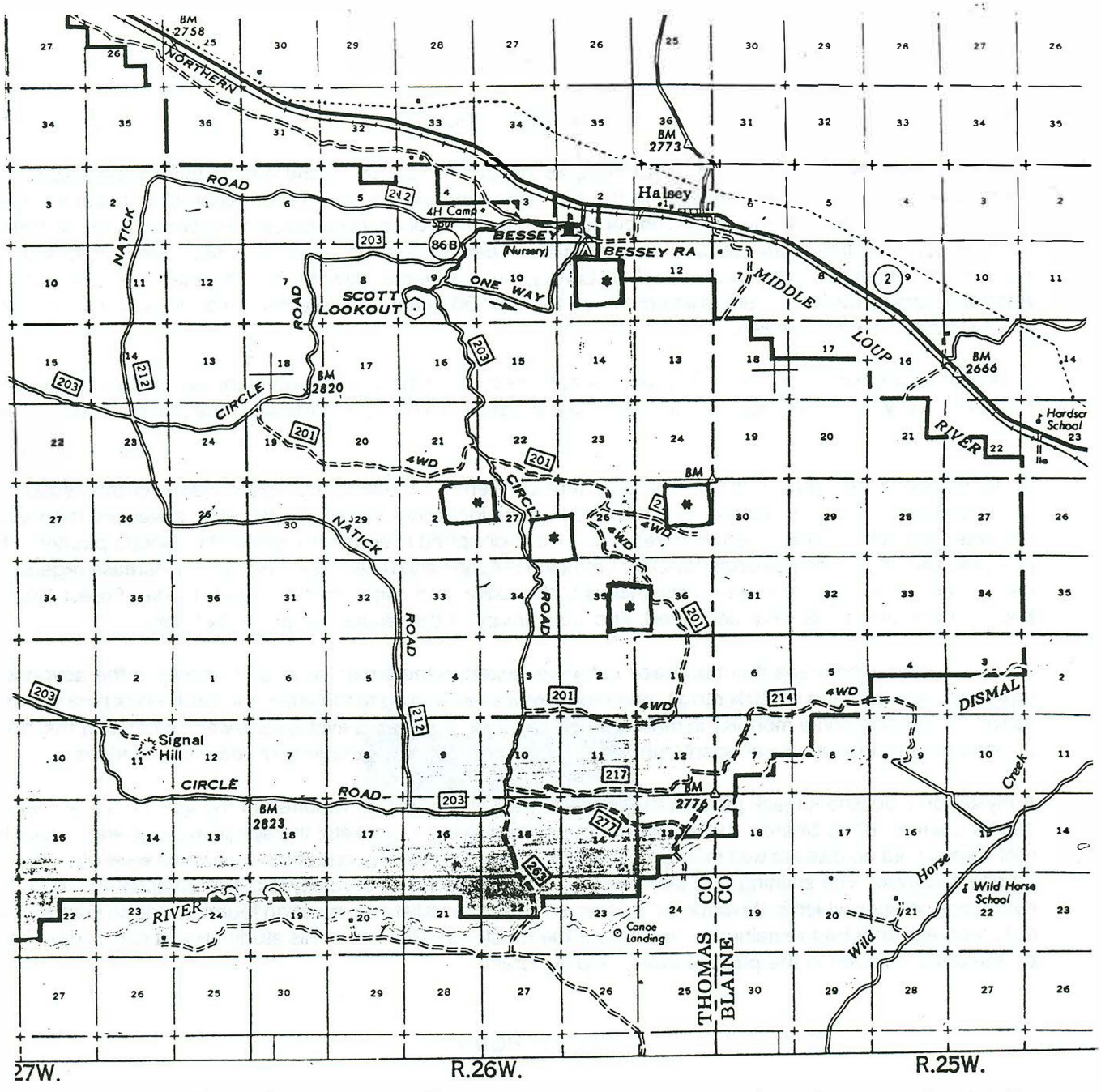

Figure 1: Locations of regeneration mortality in harvested areas.

Pitfall traps for possible beetle vectors were set up periodically near symptomatic, healthy, and dead jack pines. The traps were either metal coffee cans or insulated paper cups which had a 50% ethanol/50% turpentine solution and/or a small amount of soapy water at the bottom. The cans or cups were buried so that the opening was flush with the soil surface. Traps were set out in the late afternoon and retrieved the following morning.

For the pathogenicity tests in 2-0 jack pine, three isolates of *Leptographium terebrantis* were grown on weak malt agar. When the fungus started to sporulate, sterile toothpicks were placed on the plates. Within 2-3 weeks the fungus had actively colonized the toothpicks and was sporulating from them. The 2-0 jack pine seedlings were inoculated with either the colonized toothpicks or sterile controls in a randomized design. Ninety nine seedlings, grown in the field at Bessey Nursery, were inoculated by placing the inoculated or sterile toothpick against a small slit in the bark made immediately below the soil surface. Inoculation methods were based on Wingfield (1986). The toothpicks were then secured to the seedlings with parafilm.

Half of each of the fungus and control inoculated seedlings were slightly wounded near the inoculation point in order to simulate insect feeding. Half of the seedlings were also set apart for a low watering regime in order to achieve physiological stress. However, because the summer of 1993 was abnormally wet, this treatment could not be differentiated. Thus, the wounding was the only difference in treatments.

After approximately 11 weeks, the inoculated seedlings were pulled up, examined, and reisolations were attempted using the selective media.

RESULTS AND DISCUSSION

The areas where regeneration mortality were severe are shown in Figure 1. All areas had been harvested with an overstory removal within the past 15 years.

Symptoms on the affected regeneration and mature trees were the same. Affected trees had decreased needle retention; overall chlorosis; decreased shoot elongation; blackened, pitchy soil around the root collar; extreme resinosus in the roots and lower bole; a jet black streaky stain in the roots and lower bole; and excessive insect feeding in the roots, lower bole, and, occasionally, the stem and branches. Signs of possible causal agents were turpentine beetle pitch tubes (large pink pitch tubes when fresh, white and granular on the ground when old) on the lower bole of all affected trees, extensive root feeding beetle galleries of (probably) *Hylastes* sp., and Leptographium-like fruiting fungus in stained turpentine beetle and *Hylastes* sp. galleries.

Cores were taken from affected trees, stored in water, and examined in the lab for pine wilt nematode. There were not any nematodes found from the cores and nematodes were eliminated as a possibility for causal agents.

Isolations from the stained roots on selective medium were identified by Tom Harrington, Plant Pathologist, Department Head at Iowa State University, as *Leptographium terebrantis*, or possibly a related fungus in the terebrantis-group (see Zambino & Harrington, 1992). The same fungus was isolated from (probable) *Hylastes* sp. beetles collected from pitfall traps in affected areas.

In mid-September of 1992, 26 *Hylastes* sp. and 5 red turpentine beetles (*Dendroctonus valens* LeConte) were collected over a 2-day period from 16 pitfall traps located in two affected areas. A small sample of the beetles was sent to Steve Krauth at the University of Wisconsin and were identified as *Hylastes porculus* and *Hylastes gracilis*. In late May of 1993, a total of 14 *Hylastes* sp. were collected in one night from about 20 small pitfall traps in affected areas. A very large trapping effort was undertaken in mid-August of 1993, but with little success. Very few beetles of any kind were collected in any area. The failed trapping may have been due to recent cool, rainy weather in the area.

Several of the live root-feeding beetles were kept in jars with unstained pine twigs to see if stain was transmitted to the wood. A blue-stain like fungus was successfully transmitted in most instances; however, the fungus was not successfully isolated. Because the beetles are carriers of a variety of staining fungi, no clear conclusions can be drawn on whether or not the transmitted stain was *L. terebrantis* without positive isolates.

Several root systems were excavated and examined for insect activity. All affected trees had excessive feeding on the roots and bole by turpentine and *Hylastes* sp. beetles. Although the level of feeding was high, there was not enough activity to have completely girdled roots and caused tree mortality (Schaupp, pers. observ.). All affected trees had roots with large, stained, resiny lesions, often in association with the insect feeding.

Though *L. terebrantis* has been reported on many pine species (see Harrington & Cobb, 1988), there were no published reports found which demonstrated pathogenic capacities on jack pine. The small pathogenicity trial in the 2-0 jack pine at Bessey Nursery indicated that the fungus can be pathogenic on jack pine. Seven of the fungus inoculated seedlings (79 seedlings total) died; all were heavily stained, and the fungus was successfully reisolated from one of the dead seedlings. Six of the inoculated seedlings were showing visible signs of being infected (chlorosis and stunted growth) and the fungus was successfully reisolated from two of these seedlings. Nearly all the fungus- inoculated seedlings developed a pitchy canker response, whereas control seedlings did not have resinosus associated with the sterile toothpick inoculation. Two of the control seedlings (20 seedlings total) died, one from mechanical damage and one from unknown causes. There was no difference in results between seedlings that had been wounded to simulate insect feeding and those that had not.

Many of the reisolation attempts were quickly contaminated and it appears as though the stock solution of streptomycin used was no longer viable. Also, most of the dead seedlings had died within 6 weeks of the inoculations which may have allowed enough time for the fungus to die out before reisolations were attempted at the end of the 11 week period. Due to the excessive rainfall during that summer of 1993, the seedlings had better than optimal growing conditions, which normally would not be the case in that region, and may have contributed to the low overall mortality.

Though *Leptographium terebrantis* has been shown to be pathogenic on ponderosa pine (Harrington & Cobb, 1983; Owen et al., 1987), white pine (Wingfield, 1983; Wingfield, 1986), Japanese black pine and Scots pine (Highley & Tattar, 1985; Rane & Tattar, 1987), and is associated with red pine decline in Wisconsin (Klepzig et al., 1991), it is generally not regarded as an important pathogen. This may be because it is frequently associated with extensive insect activity, usually with the black or red turpentine beetle, and, unlike *L. wageneri*, trees must be wound inoculated to elicit a pathogenic response. Compared to *L. wageneri*, there have also been few studies done on this fungus, which has only been recently recognized by forest pathologists. The pathogenicity trial in this report does demonstrate the pathogenic potential of *L. terebrantis* on jack pine.

Although *Ceratocystis*, observed by James (1979) and Sharon (1984), is certainly abundant on most trees examined with any level of beetle activity, it most likely is not the primary cause of the observed jack pine mortality. It seems possible that without the use of the selective media, that the more pathogenic staining species such as *L. terebrantis* may have been overlooked by other pathologists. Most of the trees examined in this current study did not have significant *Ips* beetle activity as observed in earlier investigations. Some areas where the earlier decline were studied matched up closely with the areas that had been recently clearcut and were currently having extreme mortality in the advanced regeneration. When other mature stands were visited that had been described in the previous reports, the same complex was found but the mortality was not nearly as severe. In fact, most mature areas of jack pine seemed to have some level of this disease complex present, but in most cases it appeared to be spreading much more slowly as compared to the harvested areas.

CONCLUSIONS

Evidence suggests that large populations of root-feeding beetles, perhaps reaching epidemic proportions after a harvesting period, which are able to vector a potentially pathogenic *Leptographium*, are playing a large role in the dynamics of this new disease complex in the area. Whether or not this disease situation is related to complexes seen in other areas, such as the red pine decline in Wisconsin, remains to be seen. This disease complex is occurring in a very unique area, a forest planted in the Nebraska sandhills in the early 1900's. The population dynamics of the beetle vectors, and perhaps the associated fungi, may be quite different from what we would consider 'normal.' It may be possible that epidemic levels of root-feeding beetles are able to sustain themselves for prolonged periods of time due to lack of natural enemies and/or a combination of environmental and host factors. It seems likely that the jack pine stands throughout this area may not be able to sustain themselves over long periods of time due to this complex. This complex appears to be present throughout much of the jack pine stands, albeit at low levels, and attempts at cutting and future regeneration may prove quite difficult. Suggestions for management include: restricted harvest activity; push-over logging and/or stump sanitation procedures (stump removal followed by burning); and, perhaps the most feasible option, replanting with species other than jack pine and Austrian pine, such as junipers and possibly ponderosa pine (which did not appear to be affected on the Bessey Ranger District).

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